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THE EFFECT OF LOCAL CHANGES IN POTASSIUM AND BICARBONATE CONCENTRATION ON HYPOTHALAMIC BLOOD FLOW IN THE RABBIT

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SUMMARY

1. Blood flow has been measured locally in the hypothalamus of anaesthetized rabbits by measuring the clearance of small volumes (5–20 μl.) of a mock cerebrospinal fluid solution containing ^{133}Xe. The effect of varying the $[K^+]$ or $[HCO_3^-]$ of the ^{133}Xe-containing solution on local hypothalamic blood flow has been investigated.

2. There was an increase in local hypothalamic blood flow if the ^{133}Xe-containing solution was HCO_3^--free; raising the $[HCO_3^-]$ of the solution to 40 mM caused a fall in local blood flow.

3. There was an increase in local hypothalamic blood flow when ^{133}Xe was injected in a mock cerebrospinal fluid containing 10 or 20 mM-$[K^+]$. There was no significant change in blood flow if a K^+-free or a 40 mM $[K^+]$ solution was used.

4. The decrease in hypothalamic blood flow caused by injecting a 40 mM-$[HCO_3^-]$ solution could be reversed by the addition of 20 mM $[K^+]$ to the solution. There was no further increase in blood flow if 20 mM-$[K^+]$ was added to a HCO_3^--free solution.

5. It is concluded that local blood flow in the hypothalamus changes as a result of variation in local $[K^+]$ as well as local $[HCO_3^-]$. The changes in blood flow in the brain which accompany neuronal activity could be mediated by variation in local $[K^+]$.

INTRODUCTION

It has been widely accepted that one of the major determinants of the level of cerebral blood flow (CBF) is the pH of brain extracellular fluid (ECF) close to cerebral arterioles. Changes in blood flow consequent upon changes in cerebral metabolism may well be effected, therefore, through changes in local pH. The view that brain ECF pH affects local blood flow rests mainly on indirect evidence; CBF correlates poorly with arterial pH

(Harper & Bell, 1963), but well with arterial P_{CO_2} (Harper & Glass, 1965); this is compatible with the view that the ECF pH in brain is the important variable. Further evidence has been derived from experiments in which CBF has been correlated with cortical extracellular pH in cats (Betz & Heuser, 1967) or with lumbar cerebrospinal fluid (c.s.f.) pH in man (Fencl, Vale & Broch, 1969).

There are three main pieces of direct evidence which suggest that CBF is affected by local pH within the brain. Siesjö, Kjällquist, Pontén & Zwetnow (1968) measured CBF in two anaesthetized dogs during ventriculo-cisternal perfusion with a mock c.s.f. of varying bicarbonate concentration [HCO_3^-]; they found that CBF varied with the pH of the perfusate. Pannier, Weyne, Demeester & Leusen (1972) measured local blood flow in the caudate nucleus and total CBF in the cat during ventriculo-cisternal perfusion with solutions of varying pH; caudate blood flow varied with the pH of the perfusate but not cortical flow. This evidence is of value since it applies to blood flow in brain parenchyma. The third piece of evidence concerns the observed changes in pial arteriole diameter when local changes in [HCO_3^-] were produced by injections of appropriate solutions using a micropipette technique (Wahl, Deetjen, Thurau, Ingvar & Lassen, 1970). Pial arterioles constrict in the presence of a high [HCO_3^-] and dilate when the local [HCO_3^-] is reduced below the normal; the findings of Wahl *et al.* (1970) have been confirmed by Cameron & Segal (1972) using the same technique. There are certain disadvantages in this type of experiment; first the local P_{CO_2} is unknown and difficult to control and second, the pial vessels are not the site of the main cerebral vascular resistance (Stromberg & Fox, 1972).

Influences other than local pH may be of importance in regulating CBF. Recently, the possible role of other vasoactive ions, notably K^+, has been questioned. Evidence derived from experiments on pial arterioles using the micropipette technique indicates that local changes in [K^+] can affect pial arteriolar diameter; Kuschinsky, Wahl, Bosse & Thurau (1972) found that an increase in [K^+] caused a vasodilatation. Cameron & Segal (1972) using a similar technique found evidence for an interaction between changes in [K^+] and [HCO_3^-] on pial vessels, but concluded that an increase in local [K^+] could cause vaso-constriction. An effect of [K^+] on CBF could be of some importance. It has been shown that during neuronal activity, the [K^+] in the extracellular clefts in the leech central nervous system may rise as high as 14 mM (Baylor & Nicholls, 1969). If, therefore, pial vessels dilate in response to an increase in [K^+] of this magnitude and if parenchymal vessels respond similarly to pial vessels, this might represent a mechanism whereby changes in CBF could be matched to an increase in neuronal activity.

Cranston & Rosendorff (1971) developed a technique for measuring blood flow in the hypothalamus by means of the clearance of locally injected ^{133}Xe. This technique has the advantage that it measures blood flow locally within brain parenchyma; moreover, the ^{133}Xe can be injected in a solution of varying ionic composition. This technique has been used therefore, to investigate the effect of local changes in $[K^+]$ and $[HCO_3^-]$ on hypothalamic blood flow (HBF). Some of these findings have already been briefly communicated (Caronna & Cameron, 1975).

METHODS

Experiments were performed on seventy-two adult New Zealand White rabbits. Seventy animals were anaesthetized with intravenous pentobarbitone (30 mg kg^{-1}) paralysed with gallamine and artificially ventilated. Supplementary doses of anaesthetic were administered throughout the experiment. Hypothalamic blood flow (HBF) was measured by the method described by Cranston & Rosendorff (1971) for conscious rabbits. A headplate was fitted to the skull of the anaesthetized rabbit and stereotaxic co-ordinates (Monnier & Gangloff, 1961) were used to place the tips of 2 injection cannulae (tip diameter 0·4 mm) in the hypothalamus on either side of the mid line. Each cannula was connected by a 40 cm length of 00 nylon tubing to a syringe. The injection system was filled on one side with ^{133}Xe dissolved in normal rabbit mock c.s.f. (Davson, 1947) and on the other with ^{133}Xe dissolved in a test c.s.f. differing from the control in either $[K^+]$ or $[HCO_3^-]$: the control mock c.s.f. had the following composition: NaCl 125 mM, $NaHCO_3$ 25 mM, KCl 3 mM, NaH_2PO_4 1 mM, $CaCl_2$ 1 mM and $MgCl_2$ 1·5 mM. The mock c.s.f. was prepared daily by mixing appropriate stock solutions with an equal volume of ^{133}Xe-saline solution (5 mc $ml.^{-1}$). The $[K^+]$ of the solutions used for injecting the ^{133}Xe was either 0, 3, 10, 20 or 40 mM and the $[HCO_3^-]$ 0, 25 or 40 mM. The osmolality of the solutions used was maintained constant in spite of variations in $[K^+]$ and $[HCO_3^-]$ by appropriate changes in $[Na^+]$ and $[Cl^-]$. After each experiment $[Na^+]$, $[K^+]$ and osmolality of the solutions used were determined to confirm that the final composition of the solution was correct; if this was not so the results from that experiment were rejected. In some cases the $[HCO_3^-]$ of the solution was confirmed by measuring the pH of the solution after it had been equilibrated for 30 min at 38° C with a gas mixture of known CO_2%; $[HCO_3^-]$ was calculated using the Henderson–Hasselbalch equation.

The volume of ^{133}Xe-mock c.s.f. injected into the hypothalamus for each flow measurement varied from 5 to 20 μl. In any rabbit the same volume was used on either side of the hypothalamus; the larger volumes were necessary in experiments when the ^{133}Xe had decayed. The clearance of ^{133}Xe was monitored by an external collimated scintillation counter (EKCO electronics, Type M5401A) in series with an amplifier, pulse height analyser, linear rate meter (EKCO electronics) and a pen-recorder. HBF was calculated from the first 2–3 min of the semi-logarithmic plot of the clearance curve, after subtraction of background activity, using the following equation:

$$\text{HBF} = \frac{100\,\lambda \log_e^2}{T^{\frac{1}{2}}} \text{ ml. } 100\ g^{-1}\ min^{-1}.$$

Where $T^{\frac{1}{2}}$ is the half-decay time in minutes of the clearance curve, and $\lambda = 0{\cdot}74$, the brain–blood partition coefficient for the rabbit hypothalamus (Rosendorff & Luff, 1970).

HBF was measured by alternate injections of ^{133}Xe-control c.s.f. into one side of the hypothalamus and ^{133}Xe-test c.s.f. into the other side. Sufficient solution was injected to produce 2500–5000 counts sec^{-1}; since background averaged 500 counts sec^{-1} the peak to background ratio varied between 5 and 10:1. A peak above 5000 counts sec^{-1} was avoided to reduce errors due to coincident counts. At least 10 min were allowed between injections so that the counting rate returned to background. The control and test sides for injection were varied from rabbit to rabbit. HBF was measured an equal number of times on each side (usually four estimates of HBF were made on each side); for each animal, therefore, a mean test and a mean control value for HBF were obtained. The significance of the difference between control and test HBF within each group of rabbits was assessed using Student's t test for paired results. Differences in HBF between groups of rabbits were assessed using Student's t test for unpaired results. In five rabbits the control solution was injected into both sides of the hypothalamus to test any side to side variation.

During the experiment, mean arterial blood pressure was measured with a mercury manometer connected to a femoral artery cannula which was also used for obtaining anaerobic samples for pH and P_{CO_2} determinations. Blood was also withdrawn in some experiments for measuring plasma electrolytes. Rectal temperature was monitored throughout the experiment and a heating pad was used to maintain rectal temperature between 37 and 39° C.

In all rabbits the change in HBF with variation of arterial P_{CO_2} was determined at the end of the experiment during inhalation of a 10% CO_2 mixture (21% O_2:69% N_2). If in any rabbit, HBF failed to increase by at least 50% on both sides, the results from that rabbit were discarded. All rabbits were killed at the end of the experiment and 5–10 μl. carbon ink was injected through each cannula. The brains were removed, fixed in formol saline and sectioned to confirm the site of injections into the anterior hypothalamus. Any experiment in which there was evidence of local bleeding or the cannula was not in the anterior hypothalamus was rejected.

In three rabbits HBF was measured on one side of the hypothalamus (left) with ^{133}Xe in control c.s.f. while arterial blood pressure was varied. Blood pressure was altered either by I.V. infusion of angiotensin II amide (3 μg ml.$^{-1}$) until the desired level of hypertension was reached; or hypotension was produced by bleeding. Each new level of arterial blood pressure was maintained for at least 2 min before HBF was measured. The arterial P_{CO_2} of the three rabbits was adjusted either by inhalation of CO_2 or by hyperventilation, so that one rabbit was normocapnic, one hypercapnic and one hypocapnic throughout the experiment. Since, in these three rabbits, there was no cannula in the right side of the hypothalamus, the brains were removed, fixed and stained with haematoxylin and eosin to assess the local tissue damage on microscopic examination (no carbon ink was injected).

HBF was also measured in two conscious rabbits. At least 4 weeks before the experiment, a headplate was fixed to the skull under anaesthesia; the animal was then allowed to recover. During the experiment, the rabbit was restrained in a rabbit box and its head stabilized by resting on a shaped wooden block. HBF was measured in the conscious rabbit as described by Cranston & Rosendorff (1971) using ^{133}Xe dissolved in a pyrogen-free sterilized mock c.s.f. During the experiment, arterial blood for measurement of P_{CO_2} was obtained anaerobically from a cannula introduced into the central artery of an ear.

Methods of analysis

Blood for estimation of arterial P_{CO_2} and pH was withdrawn anaerobically and stored in ice slush. P_{CO_2} was measured with a Severinghaus-type CO_2 electrode (Radiometer, Copenhagen) calibrated with gases of known CO_2 concentration

analysed with the Lloyd-Haldane apparatus (Lloyd, 1958). Arterial pH was measured with a glass electrode (Radiometer, Copenhagen) calibrated with buffers of known pH values, 6·840 and 7·383 (Radiometer, Copenhagen).

[Na^+] and [K^+] were measured on samples of plasma or mock c.s.f. using a flame photometer (Instrumentation Laboratories, model 343). Osmolality was measured by depression of freezing point in an osmometer (Advanced Instruments, model 3L). All results are expressed as the mean ± S.E. of the mean.

RESULTS

In five rabbits, blood flow was measured with the cannulae tips placed in the right and left side of the hypothalamus; ^{133}Xe in normal mock c.s.f. was injected into both sides. No significant side to side variation was found (Fig. 1); this confirms the results of Cranston & Rosendorff (1971). In any experiment in which the composition of the mock c.s.f. containing

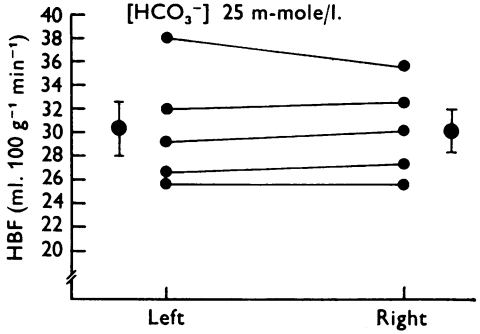

Fig. 1. A comparison of blood flow in the left and right side of the hypothalamus in five rabbits. Each point represents the mean of four measurements. There is no difference between HBF on the left and right side (left, 30·4 ± 2·2; right, 30·4 ± 1·8 ml. 100 g^{-1} min^{-1}, means ± S.E. of mean). Vertical bars in this, and in all other Figures indicate ± S.E. of mean.

the dissolved ^{133}Xe was varied, one side of the hypothalamus was used for the control injection and the other for the test. 225 paired measurements of test and control HBF were made in fifty-seven rabbits. The mean control HBF (i.e. ^{133}Xe dissolved and injected in a mock c.s.f. containing K^+ 3 mM and HCO_3^- 25 mM) was 28·5 ± 0·5 (± S.E. of mean) ml. 100 g^{-1} min^{-1}. The mean arterial P_{CO_2} during these measurements was 40·5 ± 0·5 mmHg and the mean arterial blood pressure was 84·0 ± 1·0 mmHg. The clearance curves were monoexponential, and the correlation coefficients for the linear regression analysis of the semi-logarithmic plot of the first 2–3 min of the clearance curve were always greater than 0·98. The method gives highly reproducible measurements of local flow. In the experiments shown in Fig. 1, four measurements of HBF were made on

each side of the hypothalamus in 5 rabbits over a period of 1–2 hr, the mean coefficient of variation for the measurement of HBF at the ten injection sites was 4·1 ± 0·7 %.

In twenty-seven rabbits, fifty measurements of HBF were made at levels of arterial P_{CO_2} varying from 27 to 113 mmHg; the relationship between HBF and arterial P_{CO_2} is shown in Fig. 2. The change in HBF was 0·37 ml. 100 g^{-1} mmHg P^{-1}_{a,CO_2}.

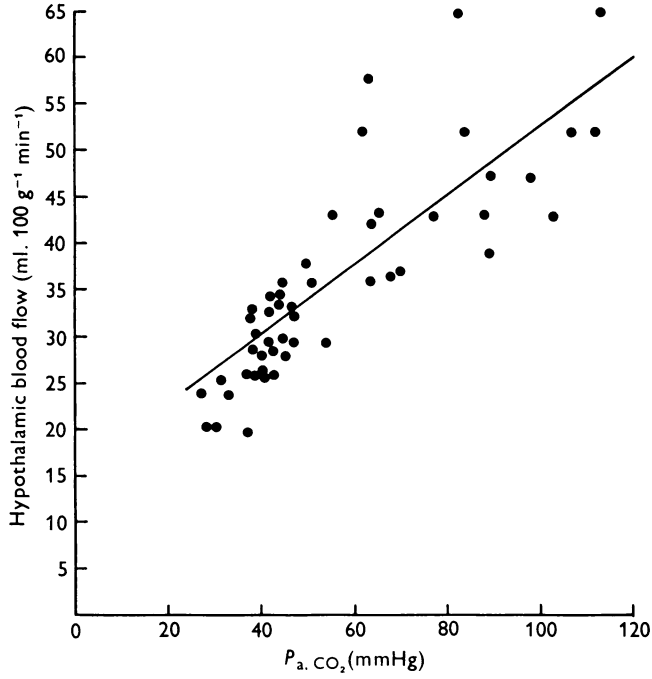

Fig. 2. The effect of varying arterial P_{CO_2} on HBF. The line drawn through the points is the least-squares regression line ($y = 0{\cdot}37x + 15{\cdot}4$, $r = 0{\cdot}83$).

In three rabbits, HBF was measured at different levels of arterial blood pressure and P_{CO_2}; HBF did not deviate from the normotensive level until mean arterial blood pressure fell below 50 mmHg, nor was there any increase in HBF with arterial blood pressure up to 140 mmHg (Fig. 3). These findings indicate that there is no side to side variation in blood flow between the right and left side of the hypothalamus, that there is an appropriate change in blood flow with variation of arterial P_{CO_2} and finally that HBF remains constant over a wide range of arterial blood pressure.

Location of the injection site and local tissue damage

The site of injection was assessed from the local staining following injection of carbon ink. In all the experiments used, the site was in the anterior hypothalamus and within 1 mm of the position predicted from the Monnier & Gangloff (1961) co-ordinate (*aB*, 16 mm). No gross damage to the hypothalamic tissue was observed in any of these rabbits. In three rabbits, the area of damage was assessed on histological examination. An area of local damage could be discerned with some inflammatory cell infiltration. The area of damage did not exceed 1 mm in diameter.

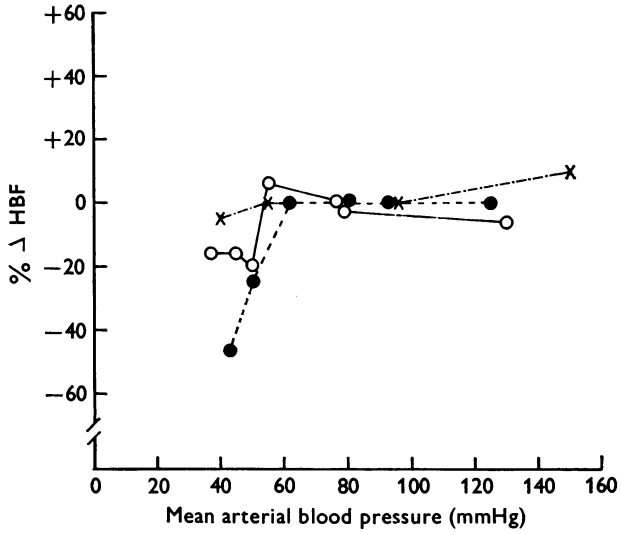

Fig. 3. The effect on HBF of varying mean arterial blood pressure in three rabbits. In each rabbit, the arterial CO_2 was maintained either at a normal (×, 39·5 ± 3·5 mmHg), raised (●, 56·9 ± 3·3 mmHg) or reduced (○, 24·9 ± 0·7 mmHg) level.

The effect of local changes in $[HCO_3^-]$ *on HBF*

The effect of local changes in $[HCO_3^-]$ on HBF was investigated by injecting ^{133}Xe dissolved in a mock c.s.f. containing either 0 or 40 mM-$[HCO_3^-]$; $[K^+]$ was maintained at 3 mM. The changes in blood flow when the solutions were injected, compared with control ($[HCO_3^-]$ 25 mM), are shown in Fig. 4. A significant increase in HBF ($P < 0·02$) was observed when the HCO_3^--free solution was used; conversely with a 40 mM-HCO_3^- c.s.f. there was a significant reduction in flow ($P < 0·005$). The solutions injected in these experiments were equilibrated at 38° C with a gas

mixture giving a P_{CO_2} of 45 mmHg and the pH measured. The pH of the 40 mM-[HCO_3^-] solution was $7{\cdot}56 \pm 0{\cdot}01$, of the 25 mM-[$HCO_3^-$] solution $7{\cdot}36 \pm 0{\cdot}01$ and of the HCO_3^--free solution $6{\cdot}35 \pm 0{\cdot}02$.

The effect of local changes in [K^+] on HBF

In another series of experiments, the [K^+] of the solution was altered while the [HCO_3^-] was maintained at 25 mM. The results of these experiments are shown in Fig. 5. There was no change in HBF when a solution

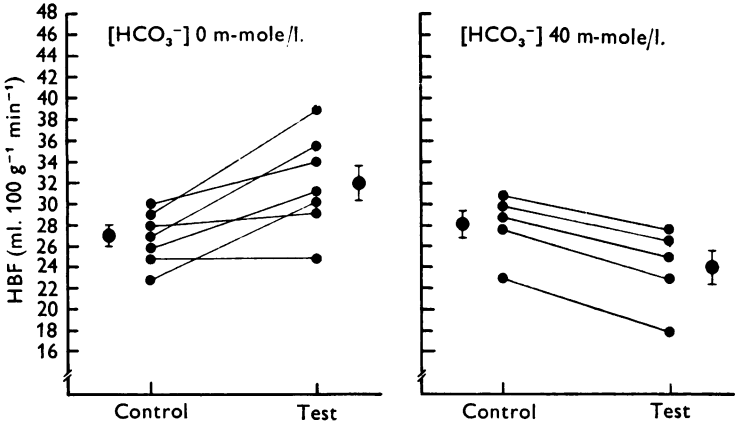

Fig. 4. The effect on HBF of varying the [HCO_3^-] of the ^{133}Xe-containing solution. HBF was reduced when the solution contained 40 mM-[HCO_3^-] (control, $28{\cdot}4 \pm 1{\cdot}3$; test, $24{\cdot}0 \pm 1{\cdot}7$ ml. 100 g^{-1} min^{-1}, $P < 0{\cdot}005$) and increased when the solution was HCO_3^--free (control, $27{\cdot}1 \pm 0{\cdot}9$; test, $32{\cdot}1 \pm 1{\cdot}7$ ml. 100 g^{-1} min^{-1}, $P < 0{\cdot}02$).

free of K^+ was used for the ^{133}Xe injection; with solutions containing an increased [K^+] of 10 or 20 mM HBF was significantly greater than control (in each case, $0{\cdot}04 < P < 0{\cdot}05$). No significant increase in flow could be demonstrated when ^{133}Xe was injected in a 40 mM-[K^+] solution ($0{\cdot}05 < P < 0{\cdot}1$).

The effect of simultaneous changes in [K^+] and [HCO_3^-] on HBF

In seven rabbits, ^{133}Xe was injected into the hypothalamus in a HCO_3^--free solution containing 20 mM [K^+]. The mean HBF was $33{\cdot}9 \pm 3{\cdot}0$ ml. 100 g^{-1} min^{-1} in these rabbits which was significantly greater than the mean control flow, $28{\cdot}0 \pm 2{\cdot}1$ ml. 100 g^{-1} min^{-1} ($P < 0{\cdot}005$). This was not greater than the HBF ($34{\cdot}7 \pm 2{\cdot}9$ ml. 100 g^{-1} min^{-1}) measured when ^{133}Xe was injected in a solution containing 20 mM [K^+] and 25 mM-[HCO_3^-] ($P > 0{\cdot}1$).

When ^{133}Xe was injected in a 20 mM-[K^+], 40 mM-[HCO_3^-] solution, there was no significant difference from control HBF (control, 29·6 ± 2·9 ml. 100 g^{-1} min^{-1}, test, 29·4 ± 2·7 ml. 100 g^{-1} min^{-1}); whereas there was a significant fall in HBF using a 3 mM-[HCO_3^-] solution. It would appear that a rise in [K^+] from 3 to 20 mM can completely overcome the vasoconstrictive effect of a 40 mM-[HCO_3^-] solution. The effects on HBF of

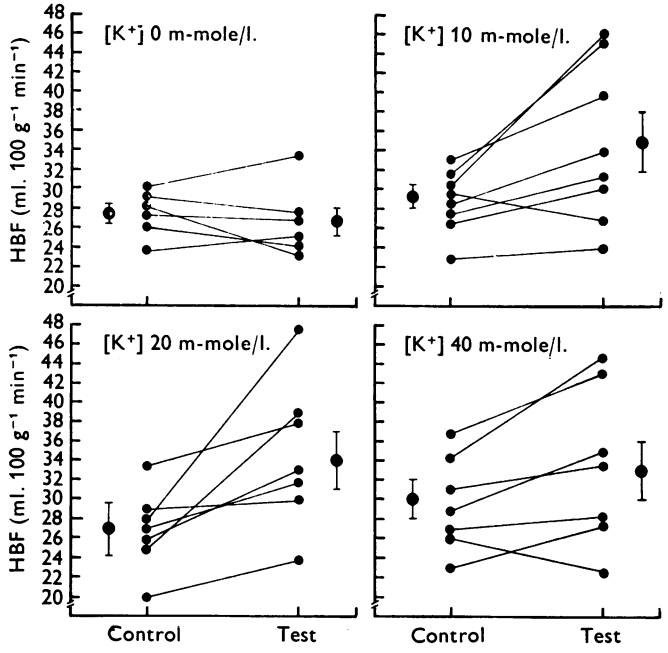

Fig. 5. The effect on HBF of varying the [K^+] of the ^{133}Xe-containing solution. There was no change in HBF using a K^+-free solution (control, 27·4 ± 0·9; test, 26·7 ± 1·5 ml. 100 g^{-1} min^{-1}, $P > 0·7$), but there was a significant increase in HBF both with a 10 mM-[K^+] solution (control, 29·3 ± 1·1; test 34·7 ± 2·9 ml. 100 g^{-1} min^{-1}, $P < 0·05$) and with a 20 mM-[K^+] solution (control 26·8 ± 1·6; test, 34·7 ± 2·9 ml. 100 g^{-1} min^{-1}, $P < 0·05$). There was no significant increase in HBF when a 40 mM-[K^+] solution was used (control, 29·6 ± 1·8; test, 33·1 ± 3·2, $0·05 < P < 0·1$).

changing local [K^+] and [HCO_3^-] separately and together are shown in Fig. 6. No further increase in HBF was observed when the [K^+] of the 25 mM-[HCO_3^-] ^{133}Xe solution was raised from 10 to 20 mM-[K^+]; nor was there any further increase in HBF when HCO_3^- was removed from a 20 mM-[K^+] solution. It appears that the maximum vasodilatory effect of an increase in [K^+] occurs at about 10 mM, and that there is no additive effect with a HCO_3^--free: 20 mM-[K^+] solution.

The effect of changes in [K+] on one side of the hypothalamus on HBF measured contralaterally

In three rabbits, HBF was measured in the usual way on one side of the hypothalamus using ^{133}Xe in the control solution; simultaneously a high [K+] solution was injected into the opposite side, this solution did not contain ^{133}Xe. In 2 of the rabbits a 40 mM [K+] solution was used and in the other a 20 mM solution. The results of these experiments are shown in Table 1; there is no indication of a generalized increase in flow throughout the hypothalamus. This evidence suggests that the changes in flow induced by the injection of a high [K+] solution are confined locally.

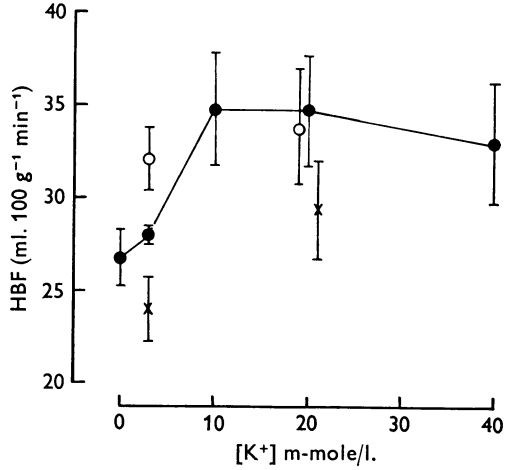

Fig. 6. The effect on HBF of varying both the [K+] and [HCO_3^-] of the solution containing ^{133}Xe. There is no increase in HBF if K^+ is increased above 10 mM, nor was there any further increase if 20 mM-[K+] and 0 mM-[HCO_3^-] were combined. ●, [HCO_3^-] 25 m-mole/l.; ×, [HCO_3^-] 40 m-mole/l.; ○, [HCO_3^-] 0 m-mole/l.

The effect of local changes in [K+] on HBF in conscious rabbits

In two rabbits, stereotaxic headplates were fitted under anaesthesia and the animals were allowed to recover. HBF was measured by injecting ^{133}Xe in a control solution and a 20 mM-[K+] solution. The results of these experiments are shown in Table 2. In both rabbits, there was an increase in HBF when ^{133}Xe was injected in a 20 mM-[K+] solution. Although the number of experiments is limited, two important points emerge; first, control HBF was 24·0 and 20·6 ml. 100 g^{-1} min^{-1} compared with the mean flow of 28·5 ± 4·0 ml. 100 g^{-1} min^{-1} in anaesthetized rabbits. The low flow in the conscious rabbits might reflect the lower level of arterial P_{CO_2} (28·7 and 27·1 mmHg) compared with the mean in anaesthe-

tized rabbits (40·5 ± 4·0 mmHg). As observed by Cranston & Rosendorff (1971) HBF differs from cortical blood flow since it is not reduced by barbiturate anaesthesia. Secondly, these experiments confirm that there is an increase in HBF in the conscious rabbit when local [K^+] is increased by

TABLE 1. Effect on HBF of injecting a high [K^+] solution contralaterally

Rabbit	[K^+] of contral. inj. mM	HBF ml. 100 g^{-1} min^{-1}					
		Control	Test	Control	Test	Control	Test
1	20	27·1	23·2	27·4	22·6	24·8	25·4
2	40	37·1	43·3	37·1	34·7	34·7	37·1
3	40	28·3	26·5	26·0	30·6	28·0	28·7

Control values were obtained by injecting ^{133}Xe in a normal mock c.s.f., test values represent a similar measurement made while a solution containing an increased [K^+] was injected contralaterally. The measurements were made in the sequence indicated.

TABLE 2. The effect of local changes in [K^+] on HBF in the conscious rabbit

Rabbit	Control HBF (3 mM-[K^+], 25 mM [HCO_3^-]) (ml. 100 g^{-1} min^{-1})	High [K^+] HBF (20 mM-[K^+], 25 mM [HCO_3^-]) (ml. 100 g^{-1} min^{-1})	Mean P_{a,CO_2} (mmHg)
1	24·0 ± 2·0	27·8 ± 2·9	28·7
2	20·6 ± 0·6	31·9 ± 0·6	27·1

Each flow is the mean ± S.E. of mean of three estimations. Mean P_{a,CO_2} is mean of measurements made at beginning and end of the experiment.

injecting 5 μl. of a 20 mM-[K^+] solution. Moreover, there was no change in the rabbits' behaviour during the period of increased HBF or during the injection; that is there was no excessive movement, change in breathing or restlessness. Since barbiturate anaesthesia would effectively control any tendency to generalized seizures after injection of a high [K^+] solution, these observations on conscious rabbits are of some importance.

DISCUSSION

Local changes in [HCO_3^-] and [K^+] alter blood flow in the hypothalamus. The increase in flow observed on injection of a HCO_3^--free solution and the decrease observed with a 40 mM-[HCO_3^-] solution provide evidence that changes in ECF pH within the brain alter local blood flow. This evidence applies to changes induced within brain parenchyma and does not depend on the use of exposed vessels. The local pH is not known in these experiments but it is reasonable to presume that in anaesthetized rabbits with a mean arterial P_{CO_2} of 40·5 ± 0·5 mmHg, the pH of the solutions

equilibrated at a P_{CO_2} of 45 mmHg will indicate the maximum changes likely to be induced in the hypothalamus. The range of pH studied was from 6·35 to 7·56, indicating that local pH as a factor affecting local blood flow may have significance under physiological circumstances. In addition, an increase in local [K^+] in the hypothalamus causes an increase in blood flow; this corroborates the findings of Kuschinsky *et al.* (1972) and does not confirm the observation of Cameron & Segal (1972) that similar changes in perivascular [K^+] cause vasoconstriction. Although an increase in [K^+] up to 10 or 20 mM caused an increase in flow, it is important to note that there was no significant increase in HBF with 40 mM [K^+]. The precise local [K^+] cannot be defined in these experiments; the [K^+] in the injected solutions represents the highest possible change induced, but diffusion of K^+ away from the site of the injection and cellular uptake presumably may reduce the local concentration. No further increase in HBF was observed if the local [K^+] was increased above 10 mM; nor was there any further increase in flow if a high [K^+] was combined with a [HCO_3^-]-free solution. This would suggest that a maximal vasodilatation is obtained either with a 10 mM-[K^+] or a [HCO_3^-]-free solution; alternatively, it is possible that the presence of high concentrations of one ion may block the action of the other, i.e. at an acid pH K^+ may no longer be vasoactive. The vasoconstriction produced by an injection of a 40 mM-[HCO_3^-] solution can be reversed by adjusting the [K^+] of the solution to 20 mM. These experiments confirm that ions other than H^+ may affect blood flow in the brain and that local changes in [K^+] may well be involved in the control of blood flow. The relative importance of local pH and [K^+] in the control of blood flow in the hypothalamus cannot be assessed from these results since the precise changes induced locally are not known.

It appears likely that the changes in flow observed are a consequence of variation in local [K^+] or [HCO_3^-] and cannot be attributed to any other consequence of the injection. The experimental design used ensures that a control mock c.s.f. is always compared with the test solution and the injections timed so that a control flow is always measured before and after each test flow. In this way there is adequate control for any alteration in the state of the animal or changes in local flow with time. The results observed cannot be attributed to variation in osmolality since the solutions used were all adjusted to have a constant osmolality and this was confirmed for every experiment. Injection of 5–20 μl. mock c.s.f. into the hypothalamus may alter local temperature but the cannulae and tubing used for injecting the test and control solutions were identical; any difference in flow between the test and control solutions cannot be attributed to variation in temperature.

The ^{133}Xe clearance method of measuring HBF has certain advantages for this type of experiment since local changes in the ionic environment can be produced simultaneously with measurement of blood flow. The method has, however, certain disadvantages; foremost among these is the damage which results from placing cannulae in the hypothalamus and injecting small volumes into the area which is to be studied. Histological examination confirms that there is local damage; in spite of this blood flow measured by this technique shows the usual features of blood flow regulation in the brain. HBF remained constant in spite of wide changes induced in arterial blood pressure and a response to changes in arterial P_{CO_2} was confirmed in all animals at the end of the experiment; any damage caused does not, therefore, compromise these aspects of local blood flow control. The mean HBF measured during the injection of the control solution was $28{\cdot}5 \pm 0{\cdot}5$ ml. 100 g^{-1} min^{-1}; this falls within the range quoted both for the conscious and anaesthetized rabbit by Cranston & Rosendorff (1971) using the same technique. HBF appears to differ from cortical blood flow as it is not only lower but does not fall during barbiturate anaesthesia; this has been observed both in the rabbit and rat (Cranston & Rosendorff, 1971; Goldman & Sapirstein, 1973) and has been confirmed in our experiments on conscious rabbits. There are no measurements of HBF in the rabbit using other techniques; Reivich, Jehle, Sokoloff & Kety (1969) measured a flow of 60–80 ml. 100 g^{-1} min^{-1} in conscious cats using a [^{14}C]antipyrine technique. There is indirect evidence which supports the conclusion that the rabbit hypothalamus has a relatively low blood flow. The tissue P_{O_2} in the rabbit hypothalamus has been found to be 78 % of cortical P_{O_2} (Cross & Silver, 1962); since tissue P_{O_2} is determined by local blood flow and metabolic rate, it must be concluded either that the hypothalamus has a low blood flow or a high metabolic rate. Rawlins, Luff & Cranston (1973) have reported that the blood content of the posterior hypothalamus is approximately 50 % of that of cortical tissue suggesting a lower vascularity. It is concluded that HBF in the rabbit may be low and that the low value cannot be attributed to local damage caused by the technique; the persistence of autoregulation and reactivity to an increased P_{CO_2} confirms that the control of blood flow in the area studied has not been destroyed.

The increase in HBF which accompanies local injection of a solution containing a high [K^+] may be due to a direct vasodilator effect of K^+; alternatively the abrupt rise in local [K^+] may increase neuronal activity or provoke an epileptiform discharge responsible for an increase in blood flow stimulated by some mechanism other than changes in [K^+]. It is known that there is an increase in blood flow during an epileptiform seizure (Plum, Posner & Troy, 1968) and that epilepsy can be produced

experimentally by increasing [K^+] locally in the brain (Zuckerman & Glaser, 1968). The changes in HBF observed in these experiments may be, therefore, a consequence of local epileptiform activity and not of a vasodilator effect of an increased [K^+]. We have no direct electrical recordings to refute this possibility but there is indirect evidence which makes such a conclusion unlikely. In the two conscious rabbits injection of a 20 mM-[K^+] solution into the hypothalamus produced no external evidence of a generalized seizure; nor was there any evidence albeit under barbiturate anaesthesia, that injection of a 20 mM-[K^+] solution into one side of the hypothalamus had any effect on blood flow on the opposite side. There is no evidence, therefore, that abrupt increases in ECF [K^+] in the hypothalamus produced generalized epileptiform discharge. Injection of 10 and 20 mM-[K^+] solutions both produced an increase in HBF; injection of a 40 mM-[K^+] solution had no significant effect on blood flow. If the changes in blood flow were secondary to an increased neuronal activity, it would be expected that a 40 mM-[K^+] solution should also produce an increase in flow. In skeletal muscle increasing [K^+] to 20 mM produces vasodilatation, whereas a further increase in [K^+] (20–30 mM) may be accompanied by vasoconstriction (Kjellmer, 1965). Vasoconstriction was not observed in our experiments at 40 mM-[K^+], nor was there a significant increase in flow compared to control. It may be that 40 mM-[K^+] is close to the concentration at which there is a reversal from a vasodilator to a vasoconstrictor action. Betz, Enzenross & Vlahov (1975) showed that exposed pial vessels dilated when a [K^+] of 10–20 mM was applied, there was little change in the diameter of the vessels at a [K^+] of 50–60 mM but at higher concentrations still vasoconstriction occurred.

In conclusion, our experiments support the view that changes in ECF [K^+] in the brain affect local blood flow, increases in [K^+] up to 20 mM being accompanied by vasodilatation. The lack of any significant increase in HBF with injection of 40 mM-[K^+] solution in our experiments, and the direct evidence of pial arteriole constriction in the presence of a high perivascular [K^+] (Betz *et al.* 1975) would support the view that blood vessels in the brain respond similarly to those in skeletal muscle in response to local changes in [K^+]; that is, by vasodilatation up to a certain perivascular [K^+], and by vasoconstriction on further increase in [K^+].

The observation that increasing [K^+] to 10 or 20 mM causes vasodilatation in the hypothalamus may have important implications for understanding the control of cerebral blood flow. Current theories assume that changes in brain ECF pH are the dominant factor in controlling blood flow in the brain (Lassen, 1968).

In the brain, neuronal activity is accompanied by an increase in blood flow (Ingvar & Risberg, 1967); there is no convincing evidence, as yet,

that under these circumstances changes in flow can be attributed to change in pH. During neuronal activity, the [K^+] in the narrow extracellular clefts in the C.N.S. of the leech may increase to 10 mM (Baylor & Nicholls, 1969). This increase in [K^+] during neuronal activity has been confirmed in cats and found to be rapid in onset, occurring in 50–300 msec (Prince, Lux & Neher, 1973). A local increase in [K^+], a direct consequence of neuronal activity, could provide an adequate stimulus for an increase in local blood flow. During normal brain activity there would be no need, therefore, to postulate a local acidosis to account for changes in blood flow.

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